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(54) Title: REVERSIBLY SEALABLE MICROSTRUCTURE SORTING DEVICES

(57) Abstract

Methods and apparatus for sorting microstructures, such as macromolecules, viruses, cells, and minute particles, in a fluid and for hermetically and reversibly sealing a microlithographic sorting array. A silicone elastomer cover is used in one embodiment. In another, silicon microstructures are used to cast elastomeric replicas of obstacle arrays, the tops of which reversibly seal against a flat surface. The reversible seal allows access to fractionated cells within the structure for further analysis.

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REVERSIBLY SEALABLE MICROSTRUCTURE SORTING DEVICES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to apparatus and methods for fractionating microstructures such as free cells, viruses, macromolecules, or minute particles. More particularly, the present invention relates to apparatus and methods for sorting such microstructures in suspension in a fluid medium, and if desired, for simultaneously viewing individual of those microstructures during the process.

2. Background Art

The sizing, separation, and study of microstructures such as free cells, viruses, macromolecules, and minute particles, are important tools in molecular biology. For example, this fractionation process when applied to DNA molecules is useful in the study of genes and ultimately in planning and the implementation of genetic engineering processes. The fractionation of larger microstructures, such as mammalian cells, promises to afford cell biologists new insights into the functioning of these basic building blocks of living creatures. One method for estimating the size of small DNA molecules is the process of gel electrophoresis.

In gel electrophoresis an agarose gel is spread in a thin layer and allowed to harden into a firm composition. The composition comprises a fine network of fibers retaining therewithin a liquid medium, such as water. The fibers of the agarose gel cross and interact with each other to form a lattice of pores through which molecules smaller than the pores may migrate in the liquid retained in the composition. The size of the pores in the lattice is determined generally by the concentration of the gel used.

Slots are cast in one end of the gel after the gel is hardened, and DNA molecules are placed into the slots. A weak electric field of typically l-l0 volts per centimeter is then generated in the gel by placing the positive pole of an electric power source in one end of the gel and the negative pole of the power source in the opposite end.

In a free solution, the mobility of a DNA molecule is independent of the length of the molecule or of the size of the applied electric field. In a hindered environment, however, aside from the structure of the hindered environment, the mobility of a molecule becomes a function of the length of the molecule and the intensity of the electric field.

The gels used in gel electrophoresis is just such a hindered environment. Molecules are hindered in their migration through the liquid medium in the gel by the lattice structure formed of the fibers in the gel. The molecules nevertheless when induced by the electric field, move through the gel by migrating through the pores of the lattice structure. Smaller molecules are able to pass through the pores more easily and thus more quickly than are larger molecules. Thus, smaller molecules advance a greater distance through the gel composition in a given amount of time than do larger molecules. The smaller molecules thereby become separated from the larger molecules in the process. In this manner DNA fractionation occurs.

The process has several inherent limitations, however. For example, the pore size in the lattice of gels cannot be accurately measured or depicted. Therefore, the lengths of the molecules migrating through the lattice cannot be accurately measured. It has also been found that DNA molecules larger than 20 megabasepairs in length cannot be accurately fractionated in gels. Apparently, the pore size in the lattice of such materials cannot be increased to permit the fractionation of larger molecules, much less even larger particles, viruses, or free cells.

Further, the lattice structure formed when a gel hardens is not predictable. It is not possible to predict the configuration into which the lattice structure will form or how the pores therein will be positioned, sized, or shaped. The resulting lattice structure is different each time the process is carried out. Therefore, controls and the critical scientific criteria of repeatability cannot be established.

Gel electrophoresis experiments cannot be exactly duplicated in order to predictably repeat previous data. Even if the exact lattice structures formed in one experiment were determinable, the structure could still not be reproduced. Each experiment is different, and the scientific method is seriously slowed.

Also, the lattice structure of a gel is limited to whatever the gel will naturally produce. The general size of the pores can be dictated to a degree by varying the concentration of the gel, but the positioning of the pores and the overall lattice structure cannot be determined or designed. Distinctive lattice structures tailored to specific purposes cannot be created in a gel.

Further, because the lattice structure arrived at depends upon the conditions under which hardening of the gel occurs, the lattice structure even in a single composition need not be uniform throughout.

Another shortcoming of gel electrophoresis is caused by the fact that a gel can only be disposed in a layer that is relatively thick compared to the pores in its lattice structure, or correspondingly to the size of the DNA molecules to be fractionated. Thus, the DNA molecules pass through a gel in several superimposed and intertwined layers. Individual DNA molecules cannot be observed separately from the entire group. Even the most thinly spread gel is too thick to allow an individual DNA molecule moving through the gel to be spatially tracked or isolated from the group of DNA molecules.

The diffusion of long polymers in complex environments where the mobility of the polymer is greatly perturbed is both a challenging statistical physics problem and a problem of great importance in the biological sciences. The length fractionation of charged polymers, such as DNA in gels, is a primary tool of molecular biology. One of the main stumbling blocks to understanding quantitatively the physical principles behind the length-dependent mobility of long polymers in complex environments has, however, been the ill-characterized nature of the hindering environment, the gel.

BRIEF SUMMARY OF THE INVENTION

It is a an object of the present invention to provide a method for hermetically and reversibly sealing a microstructure sorting device.

It is another object of the present invention to provide a method for using silicon microstructures to cast elastomeric replicas.

It is another object of the present invention to provide a method for accessing the particles inside the microfabricated structure for further analysis.

Additional objects and advantages of the invention are set forth hereinbelow in the detailed description or will be appreciated by the practice of the invention.

To achieve the foregoing objects, and in accordance with the invention as embodied and broadly described herein, the present invention is directed to a method for hermetically and reversibly sealing a microfabricated structure. The cover is removable from the microfabricated structure to allow access to the particles contained therein.

The present invention utilizes a self-sealing silicone elastomer in combination with a rigid material, such as silicon. The array may be constructed photolithographically from a material such as silicon, or may itself be formed from an elastomeric material as, for example, by casting from a silicon structure. This reversible sealing allows for access to the fractionated microstructures within the array.

Thus provided is an apparatus for sorting microstructures in a fluid medium, including a substrate having a floor bound on opposite sides by first and second side walls. The floor and the first and second side walls define a receptacle, and means are positioned within the receptacle for slowing the rate of migration of microstructures within the receptacle. A cover removably covers said receptacle. One of the cover and substrate comprise an elastomer, while the other of the cover and the substrate comprise any one of silicon, quartz, and sapphire.

Also disclosed according to the teachings of the present invention is a method of manufacturing an apparatus for sorting microstructures in a fluid medium. The method includes a step of performing a substrate having a flood bounded on opposite sides by first and second side walls with the floor and the first and second side walls defining a receptacle. In the receptacle, means are provided for slowing the rate of migration of the microstructures within the receptacle. The method further comprises the step of forming a cover and removably positioning the cover over the receptacle.

The present invention thus provides a method and apparatus that facilitates the fractionation and study of many types of microstructures. For example, the present invention allows successful fractionation of extremely long DNA molecules of chromosomal length in low quantities, such as even single molecules. The present invention also facilitates the fractionation of much larger microstructures, such as red blood cells.

It should be noted that fractionation of other macromolecules and microstructures, such a proteins, polymers, viruses, other cells, and minute particles, is also considered to be within the scope of the present invention.

It is possible, using the present invention, to generate complex environments which are very well characterized and consistently reproducible.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the manner in which the above-recited and other advantages and objects of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to a specific embodiment thereof which is illustrated in the appended drawings. Understanding that these drawings depict only a typical embodiment of the invention and are not therefore to be considered

limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 is a perspective view of one embodiment of a sorting apparatus incorporating teachings of the present invention;

Figure 2 is an exploded perspective view of the apparatus illustrated in Figure 1 with the coverslip thereof shown separated from the substrate to more fully reveal an array of obstacles therebetween;

Figure 3 is an enlarged perspective view of the obstacles within the area of the array of Figure 2 encircled by line 3-3 therein;

Figure 4 is a further perspective enlarged view of the obstacles within the area of the array of Figure 3 encircled by line 4-4 therein;

Figure 5 is a perspective enlarged view of an alternate embodiment of obstacles for an array in a sorting apparatus incorporating the teachings of the present invention to stimulate cell behavior simulating the passage of such cells through the passageways in the human body;

Figures 6A-6F illustrate the steps in a method for manufacturing a sorting apparatus, such as the sorting apparatus illustrated in Figures 1-5;

Figure 7 is a perspective view of a second embodiment of a sorting apparatus incorporating teachings of the present invention and utilizing an elastomeric cover;

Figure 8 is a cross-sectional elevation view of a peripheral portion of the array of obstacles thereof and the cover thereof disposed thereagainst;

Figure 9 is a cross-sectional elevation view similar to that of Figure 8 with the cover of the illustrated sorting apparatus lifted partially away from the array of obstacles of that apparatus;

Figures 10A-10D illustrate the steps in a method for manufacturing a sorting apparatus, such as the sorting apparatus illustrated in Figures 7-9;

Figure 11 is a perspective view of a third embodiment of a sorting apparatus incorporating teachings of the present invention and an elastomeric array of obstacles therein;

Figure 12 is a cross-sectional elevation view of a peripheral portion of the array of obstacles in the sorting apparatus of Figure 11 with the array of obstacles engaging the cover of that sorting apparatus;

Figure 13 is a cross-sectional elevation view like that in Figure 12 with the elastomeric array of obstacles thereof partially drawn away from the cover of that sorting apparatus;

Figures 14A-14D illustrate the steps in a method for manufacturing a sorting apparatus, such as the sorting apparatus illustrated in Figures 11-13; and

Figure 15 is a scanning electron micrograph perspective view of a sorting device of the type illustrated in Figures 7-9 with a reversibly sealed elastomer cover schematically shown thereon.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring to Figure 1, a sorting apparatus 20 is illustrated for fractionating and optionally for simultaneously viewing microstructures, such as free cells, macromolecules, and minute particles in a fluid medium; and doing so as desired in essentially a single layer. Sorting apparatus 20 is comprised of a substrate 22 having a shallow receptacle 24 located on a side 26 thereof. In the embodiment shown, receptacle 24 is recessed in side 26 of substrate 22, although other structures for producing a recess such as receptacle 24 would be workable in the context of the present invention.

Receptacle 24 includes a floor 28 shown to better advantage in Figure 2 bounded by a pair of upstanding opposing side walls 30, 31 and a first end 32 and a second end 34. The height of side walls 30, 31 define a depth of receptacle 24. The depth of receptacle 24 is commensurate with the size of the microstructures to be sorted in sorting apparatus 20. The depth of receptacle 24 is specifically tailored to cause those microstructures in a fluid medium in receptacle 24 to form essentially a single layer. Thus, when the microstructures are caused to migrate in the fluid medium through receptacle 24, the microstructures do so in essentially the single layer. The migration of the microstructures occurs in a migration direction indicated by arrow M defined relative to sorting apparatus 20.

Substrate 22 may be comprised of any type material which can be photolithographically processed. Silicon is preferred, however other materials, such as quartz and sapphire can also be used.

In accordance with one aspect of the disclosed technology, capping means are provided for covering receptacle 24 intermediate first end 32 and second end 34 thereof and for affording visual observation of the migration of the microstructures within

receptacle 24. As shown by way of example and not limitation, in Figure 1, a coverslip 36 extends across receptacle 24 in substrate 22 from one of the pair of upstanding opposing side walls 30 to the other of said pair of upstanding opposing side walls 31. The manner by which coverslip 36 is bonded to side 26 of substrate 22 will be discussed in detail subsequently.

According to one aspect of the disclosed technology, a sorting apparatus, such as sorting apparatus 20, is provided with means positioned within receptacle 24 for interacting with the microstructures to partially hinder the migration of the microstructures in the migration direction.

As is suggested in the exploded view of Figure 2, one form of such a means utilizable in accordance with the present invention is an array 38 of minute obstacles 39 upstanding from floor 28 of receptacle 24. Obstacles 39 are sized and separated as to advance the particular sorting objective of sorting apparatus 20. The manner of forming obstacles 39 of array 38, as well as another example of embodiments of obstacles utilizable in such an array, will be discussed in substantial detail below.

Coverslip 36 is so secured to the top of obstacles 39 in array 38 as to preclude migration of microstructures between the obstacles 39 and coverslip 36. Coverslip 36 is optionally transparent, so as to permit visual observation therethrough of the migration of microstructures through array 38. Coverslip 36 may be comprised of any ceramic material. Pyrex is preferred, but other materials, such as quartz and sapphire, for example, may also be used.

In accordance with another aspect of the disclosed technology, a sorting apparatus, such as sorting apparatus 20, is provided with electric force means for generating an electric field in the fluid medium in receptacle 24. The electric field induces the microstructures to migrate through the fluid medium, either from first end 32 to second end 34 or from second end 34 to first end 32, depending upon the polarity of the electric field and whether the microstructures are positively or negatively charged. Negatively charged microstructures, such as DNA molecules, will be induced to flow toward the positive pole. Positively charged microstructures, such as proteins, will be induced to flow toward the negative pole.

By way of example and not limitation, a first electrode 40 is shown in Figure 2 as being located in first end 32 of receptacle 24 and a second electrode 42 located in second end 34 of receptacle 24. First electrode 40 and second electrode 42 each comprise

a metal strip disposed on floor 28 of receptacle 24. In one embodiment, the metal strip is formed from evaporated gold.

A battery 44, or other power source is electrically coupled between first and second electrodes 40 and 42, such that first electrode 40 comprises a negative pole and second electrode 42 comprises a positive pole. The electric field generated between first and second electrodes 40 and 42, is non-alternating, but the use of an alternating power source in place of battery 44 would be acceptable.

When DNA is the microstructure being induced to migrate, the electric field intensity in receptacle 24 is in the range of from about 0.1 volt per centimeter to about 20 volts per centimeter.

Referring now to Figure 3, the portion of Figure 2 encircled by line 3-3 is seen illustrated in an enlarged manner. Figure 3 illustrates one example of a means for use in a sorting apparatus of the present invention. As shown, array 38 comprises a plurality of obstacles 39 upstanding from floor 28 of receptacle 24. Although Figure 3 illustrates obstacles 39 as being positioned within array 38 in an ordered an uniform pattern, it is within the scope of the present invention to have a staggered pattern, or any desired predetermined and reproducible pattern.

Figure 4 illustrates the various dimensions of a typical obstacle 39. The height H of obstacle 39 is measure in a direction normal to floor 28 of receptacle 24. The length L of obstacle 39 is measured in a direction parallel to said migration direction M. The width W of obstacle 39 is measured in a direction normal to the migration direction M. Each of the obstacles 39 are separated from an adjacent obstacle 39 by a predetermined separation distance S_d. The space between adjacent of obstacles 39 is a cross section of array 38 taken normal to floor 28 of receptacle 24 defines a pore 54 of the lattice structure cumulatively produced by obstacles 39 of array 38. For convenience of reference in Figure 4, such a typical pore 54 has been shaded.

Figure 4A, a cross-section of two obstacles 39, illustrates in planar view a typical pore 54. Pore 54 compresses the area defined by two obstacles 39 through which a microstructure must pass. Pore 54 is defined by the height H and the separation distance S_d between the obstacles. The desired size of pore 54 is determined by reference to the size of the microstructures to be sorted therethrough.

An important aspect with the apparatus of the present invention is that no only is the pore size of the arrays known, but it is also constant and reproducible.

These dimensions can be changed and designed to be as desired depending upon the type and size of microstructure to be sorted, the design of the array, and the type of obstacles in the array.

For example, the separation distance S_d will vary depending upon whether the migration of microstructures through pores 54 are DNA molecules, viruses and bacterial cells, or mammalian cells. For migration of DNA molecules, the separation distance S_d is within the range of about 0.01 microns to about 20.0 microns. For migration of viruses and bacterial cells, the separation distance S_d is within the range of about 0.01 microns to about 1.0 micron. For migration of mammalian cells, the separation distance is within the range of from about 1.0 micron to about 50.0 microns. It is presently preferred that the separation distance S_d be substantially equal to the radius of gyration of the molecule, the radius of gyration being the distance walking out from the center of the molecule.

Length L also varies depending upon the microstructure to be migrated through array 38 of obstacles 39. In a presently preferred embodiment, the length is generally equal to the separation distance. With regard to height H, the height of obstacles may generally be in the range of from 0.01 microns to about 20.0 microns. For smaller microstructures, the obstacles may have a height in a range from about 0.01 microns to about 0.50 microns. For larger microns, the height may be in the range from about 1.0 micron to about 5.0 microns.

Referring now to Figure 5, another embodiment of an array of obstacles can be seen that is particularly suitable for sorting larger microstructures, such as free cells, viruses, or minute particles.

In Figure 5, an array 60 of obstacles in the form of elongated rectangular bunkers 62 is positioned within receptacle 24. Bunkers 62 are comprised of a rectangular shape having opposing sidewalls 64 and a top 66. Bunkers 62 upstand from floor 28 of receptacle 24. Bunkers 62 are positioned within columns and rows within receptacle 24. Cells, for example, migrate through the columns and between the rows of bunkers 62 in a migration direction indicated by arrow M. The longitudinal axis of bunkers 62 is disposed in alignment with migration direction M. Channels 68 are formed between rows of bunkers 62 through which the cells migrate. A separation distance S_r between rows of bunkers 62 indicates the size of channels 68.

The size and organization of bunkers 62 may vary. Thus, the separation distance S_d may be sized to allow the cells to migrate through channels 68 in essentially a single layer in at least one single file.

The height H of each bunker 62 should also be such as to allow the cells to pass through the bunkers 62 in essentially a single layer. As with sorting apparatus 20, a coverslip 36 is fused to the tops 66 of bunkers 62 to prevent migration of cells between the coverslip and the tops 66 of bunkers 66. This ensures that the cells migrate through the array 60 of bunkers 62 in essentially a single layer.

Bunkers 62 are but examples of obstacles for forming channels 68. Different structures may also be used to simulate channels through which the cells can migrate and be observed.

The method of making apparatus of the type disclosed above involves forming receptacle 24 on one side of substrate 22. Receptacle 24 should be formed of a size such that microstructures migrate in the fluid through receptacle 24 in essentially a single layer. A further step comprises creating array 38 of obstacles 39 within receptacle 24. Each of obstacles 39 have a top 56, sides 57, and a bottom end 58. Obstacles 39 are upstanding from floor 28 of receptacle 24 in a predetermined and reproducible pattern. In one preferred embodiment, the array of obstacles comprises a plurality of posts.

By way of example and not limitation, the creation of obstacles, such as posts, or bunkers, within the receptacle is illustrated in Figures 5A-5F. As shown in Figure 5A, the forming step comprises developing a photosensitive photoresist layer 70 over areas of substrate 22 that are intended to correspond to tops 56 of obstacles 39. This is accomplished by exposing substrate 22 to light through a mask having thereon a corresponding opaque pattern.

The portion of photoresist layer 70 which is exposed to light becomes soluble in a basic developing solution, while the unexposed portion remains on substrate 22 to protect substrate 22. Thus, after development in the developing solution, substrate 22 is left with a pattern of photoresist layer 70 that is identical to the opaque pattern of the mask. Figure 5B illustrates substrate 22 with photoresist layer 70 thereon after exposure to light and development in solution.

The next step comprises etching substrate 22 such that the areas of substrate 22 unshielded by photoresist layer 70 are exposed to the etching, thereby forming

receptacle 24. The array 38 of obstacles 39 upstanding within the etched receptacle 24 is formed by the portions of substrate 22 shielded by photoresist layer 70. Figure 5C illustrates formation of receptacle 24 and the obstacles 39.

As can be seen in Figure 5C, as the substrate 22 is etched, the photoresist layer 70 is also etched, but at a slower rate. Figure 5C illustrates the receptacle 24 half formed, and photoresist layer 70 partially etched away. If, for example, the photoresist layer is etched at a rate 1/10 the rate that substrate 22 is etched, the resulting receptacle can at most have a depth 10 times the thickness of the photoresist layer. The thickness of photoresist layer 70 must therefore be chosen accordingly.

The etching process can be terminated at any time when the desired depth of the receptacle is reached. As illustrated in Figure 5D, there may be some photoresist layer 70 still present on substrate 22 when the etching is terminated. If so, the next step is then dissolving photoresist layer 70 from substrate 22. This step leaves a clean substrate 22 as shown in Figure 5E.

Etching may be effected by many methods. In the preferred embodiment, ion milling is used such that an overhead ion beam is used to etch the substrate 22 and photoresist layer 70. Other methods of etching, such as chemical etching, are also within the scope of the present invention.

The step of fusing coverslip 36 to substrate 22 is illustrated in Figure 5F as comprises positioning coverslip 36 over array 38 of obstacles 39, such that coverslip 36 is in contact with each of obstacles 39, and then applying an electric field between coverslip 36 and each of obstacles 39. The coverslip 36 is held with a negative potential. The obstacles 39 are held at a positive potential. Ions are thereby induced to migrate there between to create a bond between coverslip 36 and each of obstacles 39 at all areas of contact. The process of this step is referred to as field assisted fusion.

The voltage used to fuse coverslip 36 to the substrate 22 is preferably about 1 kilovolt but can be within the range of from 200 volts to about 2000 volts. The time for fusion is about 30 minutes at a temperature of about 400°C. The temperature can also range from about 300°C to about 600°C, with 400°C being the preferred temperature. In one embodiment, the coverslip comprises a Pyrex material. For example, sapphire, and quartz are materials which may also be used for the coverslip. Any ceramic material or an opaque material may also serve.

In the context of using field assisted fusion to secure the coverslip and substrate, it is advisable that the material used for coverslip 36 have substantially the same coefficient of thermal expansion as substrate 22. Otherwise, at the high temperature of fusion, the coverslip 36 and the substrate 22 will expand at different rates and a seal between the two would be difficult or impossible to accomplish.

Successful fusion can be tested by injecting a fluorescent fluid into the apparatus. A completely fused coverslip will not allow passage of any fluorescent fluid between coverslip 36 and obstacles 39.

The method of making the apparatus disclosed above, by involving Pyrex-silicon based anodic bonding to enclose the microstructure, entails high temperature and high voltage conditions that can damage components and make the resulting device usable only once. Furthermore, once the structure is sealed, the process is not reversible. This ultimately prevents access to the sorted particles inside the structure for further analysis or otherwise.

The present invention thus provides further methods and apparatus for fractionation of microstructures in a reversibly sealable microfabricated structure.

For purposes of brevity and simplicity, the following discussion shall be directed to the methods of the present invention for use in connection with white blood cell fractionation, but it should be understood that such discussion is merely exemplary.

Figure 7 illustrates a second embodiment of a sorting apparatus 80 that is similar in several respects to sorting apparatus 20 illustrated in Figure 1. Accordingly, structures in sorting apparatus 80 that are, for the purposes of this discussion, substantially identical to structures already identified in sorting apparatus 20 will be identified by the same terminology and reference characters as was utilized in relation to sorting apparatus 20.

Sorting apparatus 80 is comprised of an elongated substrate 82 having a correspondingly elongated receptacle 24 located on a side 26 thereof. A number of cells 81 to be fractionated in sorting apparatus 80 are shown in a first loading area 82 moving in migration direction M toward a second loading area 84 at the opposite end of receptacle 24. This movement of cells 81 occurs in a fluid medium, which is for simplicity not shown. It should be noted in addition that while first electrode 40, second electrode 42, and the battery 44 are illustrated as providing an electrophoretic-type of mobility to cells 81, these are included by way of example as but one of many structures by which microstructures could be induced to move in migration direction M. Additional

approaches to inducing migration of cells 81 might include induced fluid flow, gravity, and other techniques.

To migrate from first loading area 81 to second loading area 84, however, cells 81 pass through an array 86 of obstacles taking the form of bunkers 62 of the type illustrated in Figure 5. Sorting apparatus 80 is provided with a cover 88 that engages the tops of bunker 62 in array 86, but which in contrast to coverslip 36 illustrated in Figure 1, is both flexible and nondestructively removable from the position thereof shown in Figure 7. Typically, cover 88 is formed from an appropriate elastomer in a manner to be disclosed subsequently, and cover 88 may if desired, be rendered transparent in order to permit viewing of the migration of cells 81 through array 86. Nonetheless, cover 88 can be selectively lifted away from substrate 22 in the manner also illustrated in Figure 7, thereby affording physical access to any specific fractionated portion of the collection of cells 81 migrating through array 86.

These elements of sorting apparatus 80 are shown in the elevation cross section views of Figures 8 and 9 for enhanced clarity. In Figure 8, cover 88 is shown extending across and making contact with the top 66 of each bunker 62 in array 86. In Figure 9, a portion of cover 88 is shown uplifted from bunkers 62 to afford access to the interior of array 86.

Figures 10A-10C illustrate steps in a method of manufacturing a selectively removable cover, such as cover 88 of sorting apparatus 80. As shown in Figure 10A, a liquid elastomer 90 is deposited on a flat surface 92, which is rotated in the plane thereof as indicated by the arrows R. As a result, liquid elastomer 90 is spread on surface 92 in a sheet of uniform thickness, which is then cured.

As shown in Figure 10B, the sheet of cured elastomer 94 is then cut into a size and shape corresponding to cover 88. Finally, cover 88 is peeled out of the sheet of cured elastomer 94 and off of surface 92 to be disposed across and in contact with array 86 of bunkers 62 in the manner shown in Figures 7 and 8.

Yet a third embodiment of a sorting apparatus 100, according to teachings of the present invention, is illustrated in Figure 11. Sorting apparatus 100 comprises a rigid coverslip 36 of the type illustrated in Figure 1 in combination with a flexible substrate 102 that is selectively detachable from coverslip 36 for the purpose of affording access to array 86 of bunkers 62 disposed below coverslip 36. Typically, substrate 102 and array 86

of bunkers 62 are formed of an elastomer, of the type disclosed above as used to form cover 88 in sorting apparatus 80.

Figures 12 and 13 illustrate selected of these relationships in sorting apparatus 100. In Figure 12, rigid coverslip 36 can be seen disposed across and in contact with tops 66 of bunkers 62 in array 86. In Figure 13, a portion of flexible substrate 102 has been moved downwardly, separating tops 66 of bunkers 62 from coverslip 36.

A method of manufacturing a flexible substrate, such as substrate 102, is illustrated in Figures 14A-14D.

In Figure 14A, a rigid substrate, such as substrate 26 shown in Figure 5F, is manufactured according to the microlithographic techniques described in relation to Figures 5A-5F. Substrate 26 is not, however, utilized directly to provide an array of obstacles for use in sorting apparatus 100. Instead, substrate 22 is utilized as a mold in the manner shown in Figure 14B upon which to cast a quantity of liquid elastomer 90, which is then cured. As illustrated in Figure 14C, cured elastomer 94 is then removed from substrate 22 as shown by arrow A and upended as shown in Figure 14D, producing substrate 102 supporting array 86 of upstanding bunkers 62. Rigid coverslip 36 is received and removably sealed to array 86 as suggested by arrows B.

The microlithographic methods discussed above were used to construct arrays of obstacles of silicon. The silicon arrays were cleaned in a 1/1/1 by volume mixture of boiling distilled water, hydrogen peroxide and ammonium hydroxide for 20 minutes. 5 grams of General Electric silicone type RTV615 was mixed with catalyst and degassed in a vacuum oven, poured over a clean silicon wafer, degassed and cured at 80°C for 1 hour. The cured elastomer was placed over the array, and spontaneous sealing occurred. A diluted solution of hydrophilic polyurethane from Tyndale Plains Hunter Inc. was allowed to wet the sealed array at the open end and dried. Other polymers including hydrophilic polyether polyurethane, vinylpyrrolidone or polymers comprising acrylamide, acrylic acid, and/or hydroxyethylmethacrylate may also be used. Saline buffer was then used to wet the sealed, coated array.

The self-sealing silicone elastomer successfully reversibly sealed the tops of photolithographically constructed arrays of synthetic microfabricated channels. Use of reversible sealing covers constructed of silicone elastomers allowed access to the fractionated cells within the array.

Cells in the array did not stick to the elastomer lid which sealed the top of the array. This fact was observed by lifting the elastomer lid off the array and re-imaging the exposed cell using fluorescent. The cell density and pattern before and after removing the elastomer lid was not changed. Examination of the elastomer lid using epi-fluorescence after removal showed that no cells had adhered to the removed elastomer, confirming that the cells had specifically bonded to the polymer treated silicon structures. While adhesion of the cells to the coated silicon substrate may be due to either electrostatic interactions with the underlying silicon or cell protein/ligand binding to the polyurethane coating, the non-adhesion of the cells to the elastomer lid opens the exciting possibility that the fractionated cells can be individually removed from the array for further analysis, a crucial aspect of the removable lid technology developed herein. Exploitation of the potential of these devices for cell biology will require advances in both cell biology and understanding the physics of the deformation and adhesion of soft surfaces.

Figure 15 shows a composite picture of the array design, which has channels with lengths varying from 20 μm to 200 μm in a stepwise fashion, with etched depth and width of 5x5 μm. The arrays were sealed hermetically on their top by placing a flat rectangle approximately 1 mm thick of the silicon elastomer polydimethylsiloxane (PDMS) (Type RTV416, GE Polymers) over a clean, dry array. The reversibly sealed elastomer lid is shown schematically in this figure. Visualization of blood cells in the array was done using three techniques: (1) reflected epi-illumination, (2) epi-fluorescent imaging of cell nuclei using a DNA binding dye, benzimidazole (Hoechst dye 33342, or H33342) a vital nuclear stain, and (3) epi-fluorescent imaging of the external cell walls using fluorescent-labeling antibodies which are specific to selected type of leukocytes.

In Figure 15, arrays were fabricated on a silicon wafer using a combination of CHF_3 etching via photoresist masks and CI etching using a SiO_2 mask. The etch depth was 5 microns. Superimposed on this micrograph is a representation of the polydimethylsiloxane (PDMS) elastomer lid which seals the array by the blue-tinted rectangle, and the green-tinted section indicates that the elastomer can be reversibly lifted from the surface. The PDMS was General Electric silicone type RTV615, cast on a silicon wafer surface followed by cutting and removal from the surface. On the right hand side of the figure is an image taken epi-illumination of a leading front of red blood cells as they approach the array. Hydrodynamic pressure gradients on the order of 5lb/in^2/in

(or $3x10^3$Pa/cm) move erythrocytes easily through such a sealed array at speeds around 1mm/s if the array surfaces have been chemically cleaned.

Thus the present invention allows for the fractionation of microstructures in a reversibly sealable microfabricated structure. In particular, the reversible seal of the present invention allows access to fractionated cells within the structure. The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive.

WHAT IS CLAIMED IS:

1. An apparatus for sorting microstructures in a fluid medium, the apparatus comprising:

a. a substrate having a floor bound on opposite sides by first and second side walls, the floor and the first and second side walls defining a receptacle;

b. means positioned within the receptacle for slowing the rate of migration of the microstructures within the receptacle; and

c. a cover removably covering said receptacle.

2. The apparatus as recited in Claim 1, wherein said cover is hermetically sealed to said substrate.

3. The apparatus as recited in Claim 1, wherein one of said cover and said substrate comprises an elastomer.

4. The apparatus as recited in Claim 3, wherein said elastomer is a silicone elastomer.

5. The apparatus, as recited in Claim 4, wherein said silicone elastomer comprises polydimethylsiloxane.

6. The apparatus as recited in Claim 3, wherein the other of said cover and said substrate comprises any one of silicon, quartz, and sapphire.

7. A method of manufacturing an apparatus for sorting microstructures in a fluid medium, the method comprising the steps of:

a. forming a substrate having a floor bounded on opposite sides by first and second side walls, said floor and said first and second side walls defining a receptacle;

b. providing within said receptacle means for slowing the rate of migration of the microstructures within said receptacle;

c. forming a cover; and

d. removably positioning said cover over said receptacle.

8. The method as recited in Claim 7, wherein said cover is hermetically sealed to said substrate.

9. The method as recited in Claim 7, wherein one of said cover and said substrate comprises an elastomer.

10. The method as recited in Claim 9, wherein said elastomer is a silicone elastomer.

11. The method as recited in Claim 10, wherein said silicone elastomer comprises polydimethylsiloxane.

12. The method as recited in Claim 9, wherein said other of said cover and said substrate comprises any one of silicon, quartz, and sapphire.

13. The method as recited in Claim 9, wherein said elastomer is cast from a rigid structure.

14. The method as recited in Claim 13, wherein said rigid structure comprises any one of silicon, quartz, and sapphire.

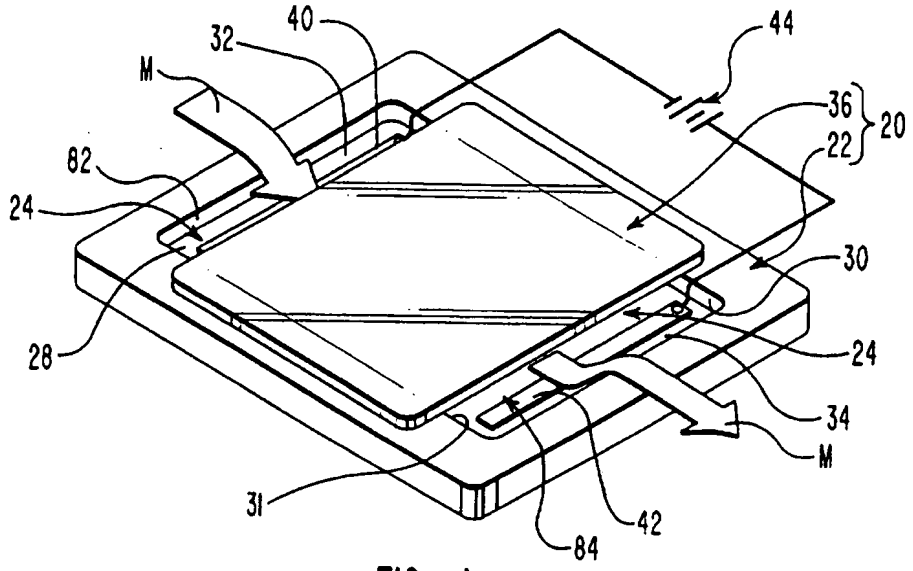
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FIG. 1

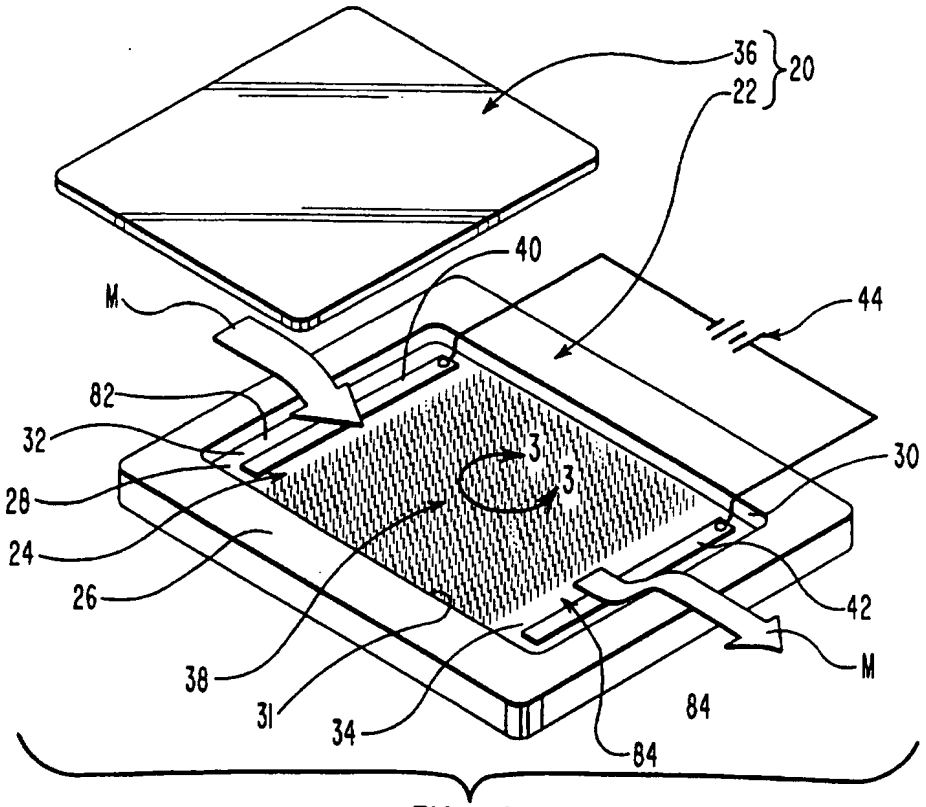
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FIG. 2

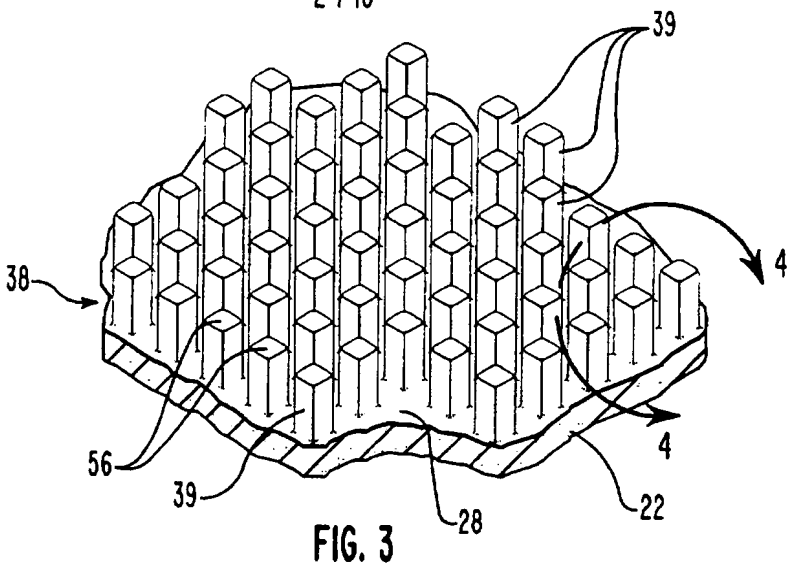
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FIG. 3

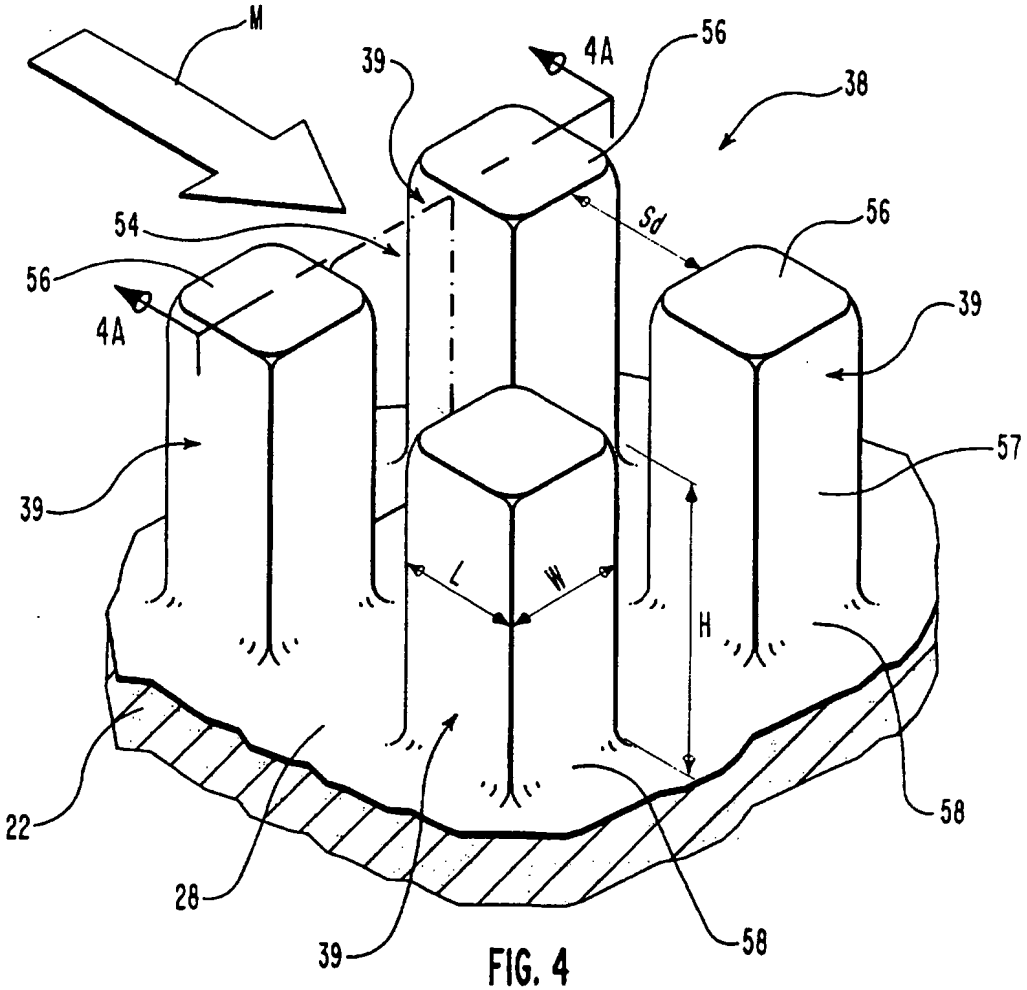
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FIG. 4

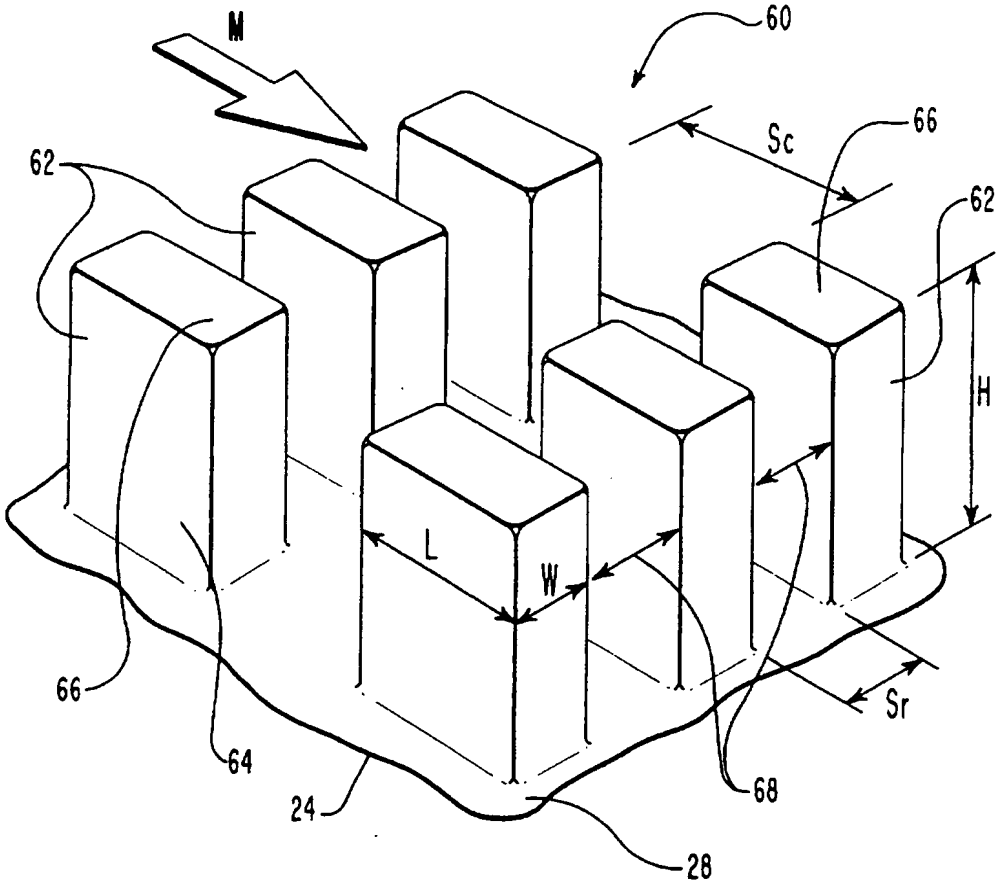

FIG. 5

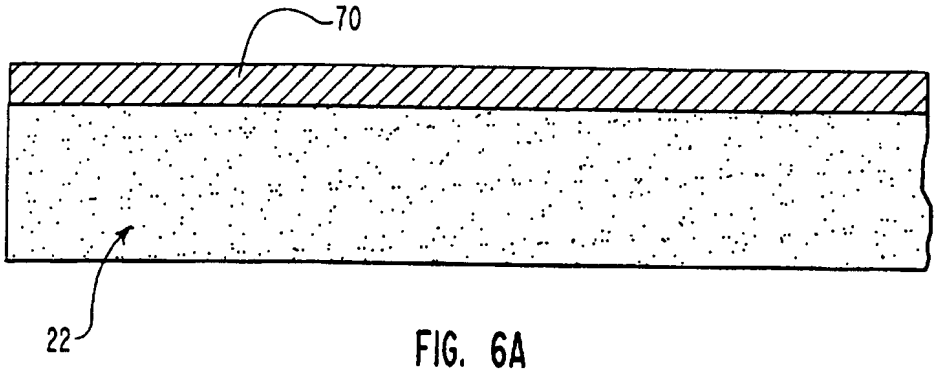

FIG. 6A

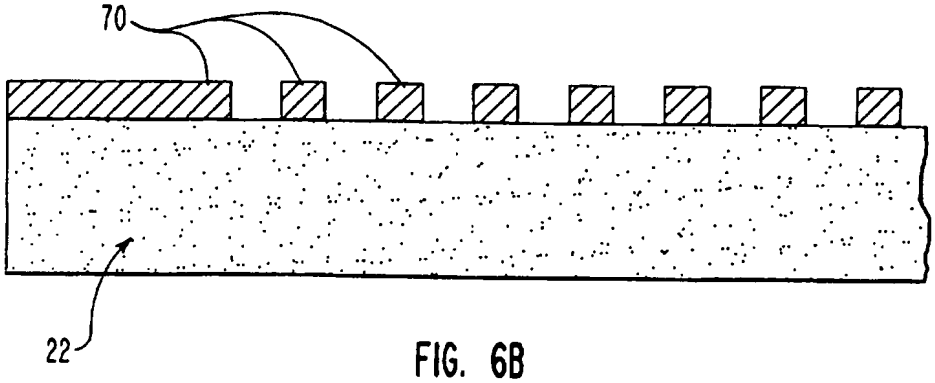

FIG. 6B

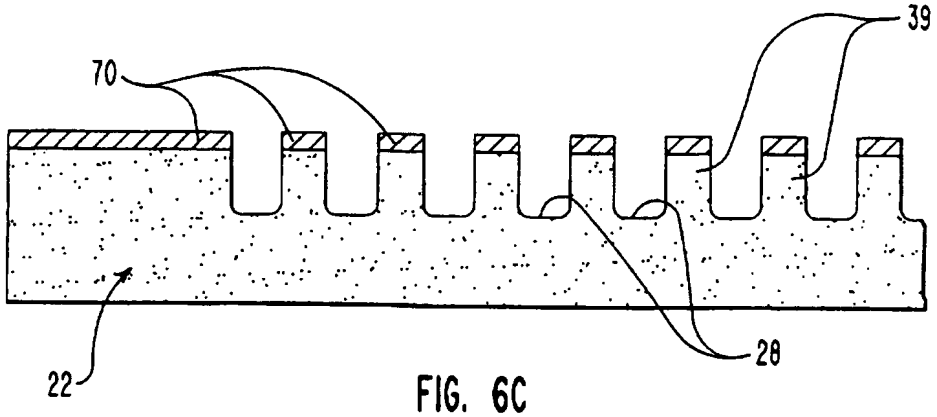

FIG. 6C

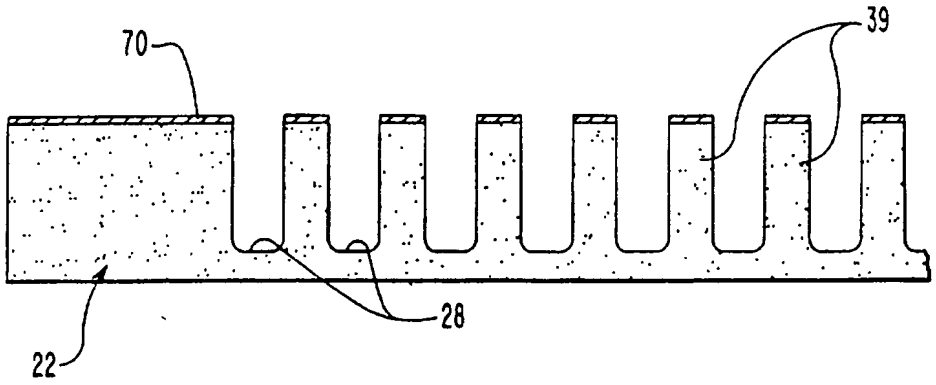

FIG. 6D

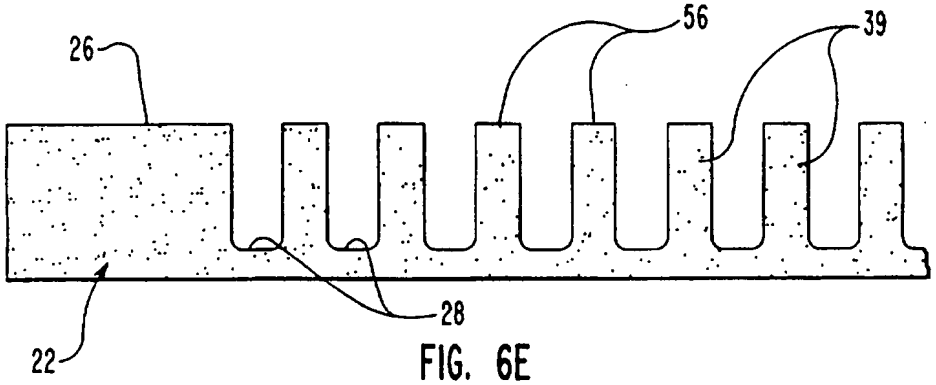

FIG. 6E

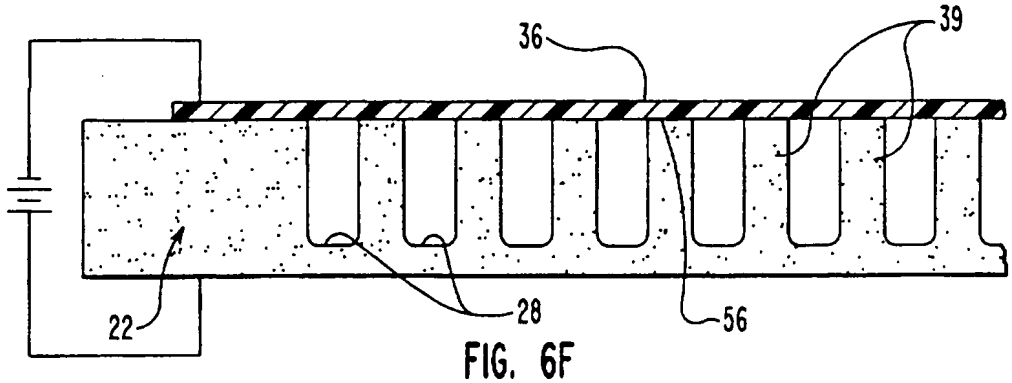

FIG. 6F

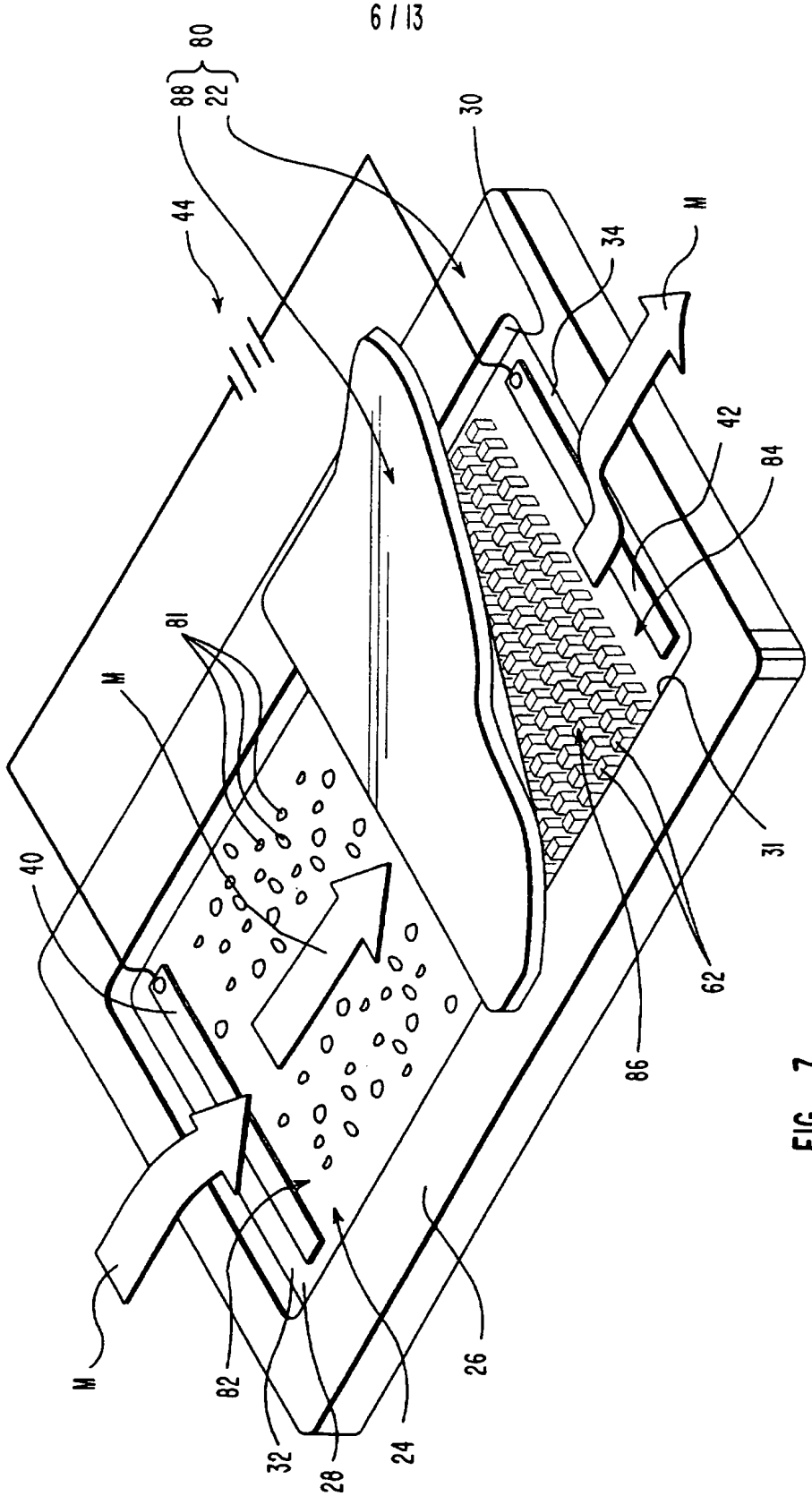

FIG. 7

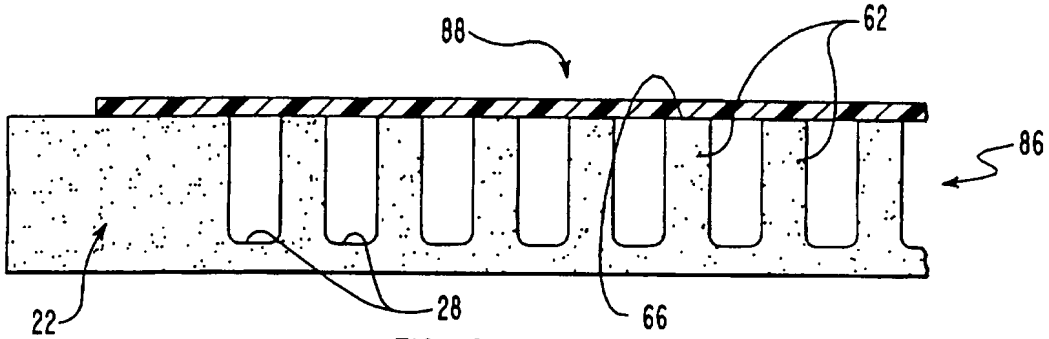

FIG. 8

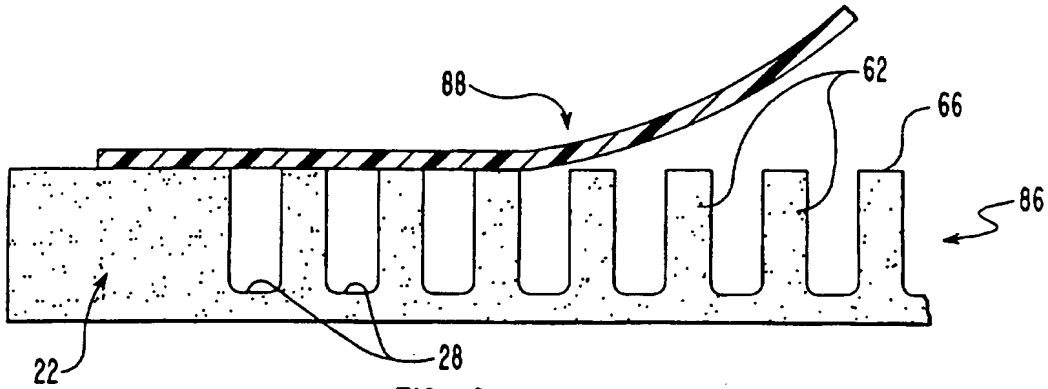

FIG. 9

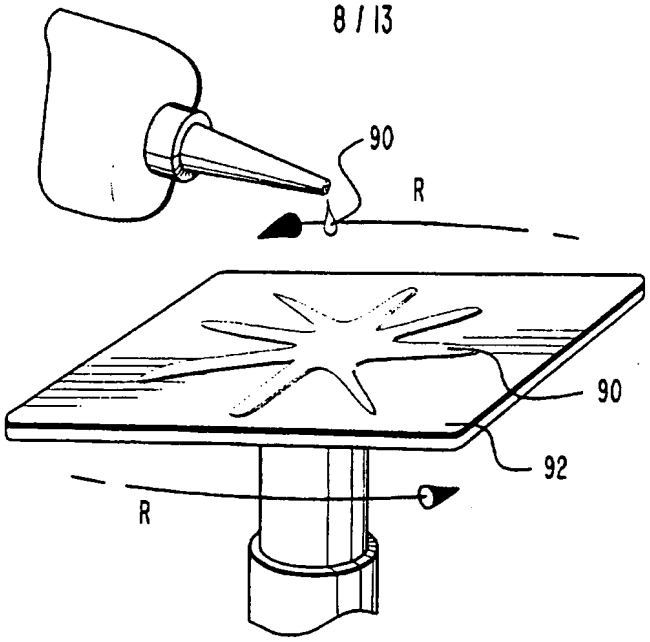

FIG. 10A

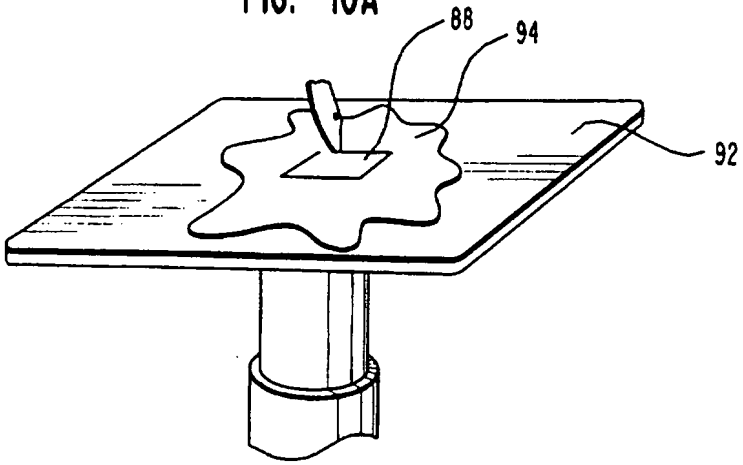

FIG. 10B

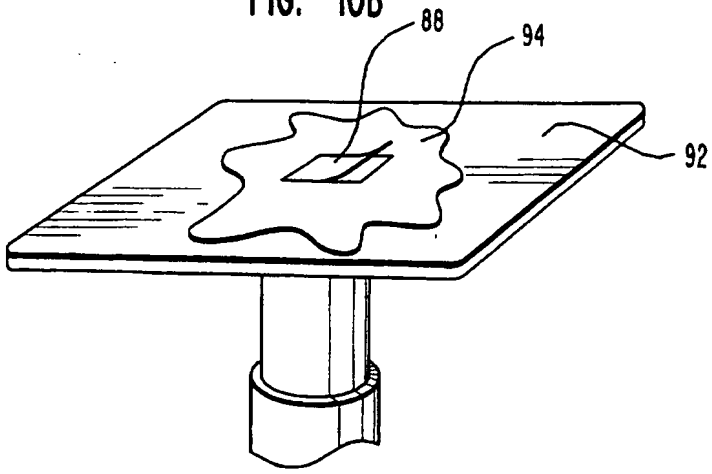

FIG. 10C

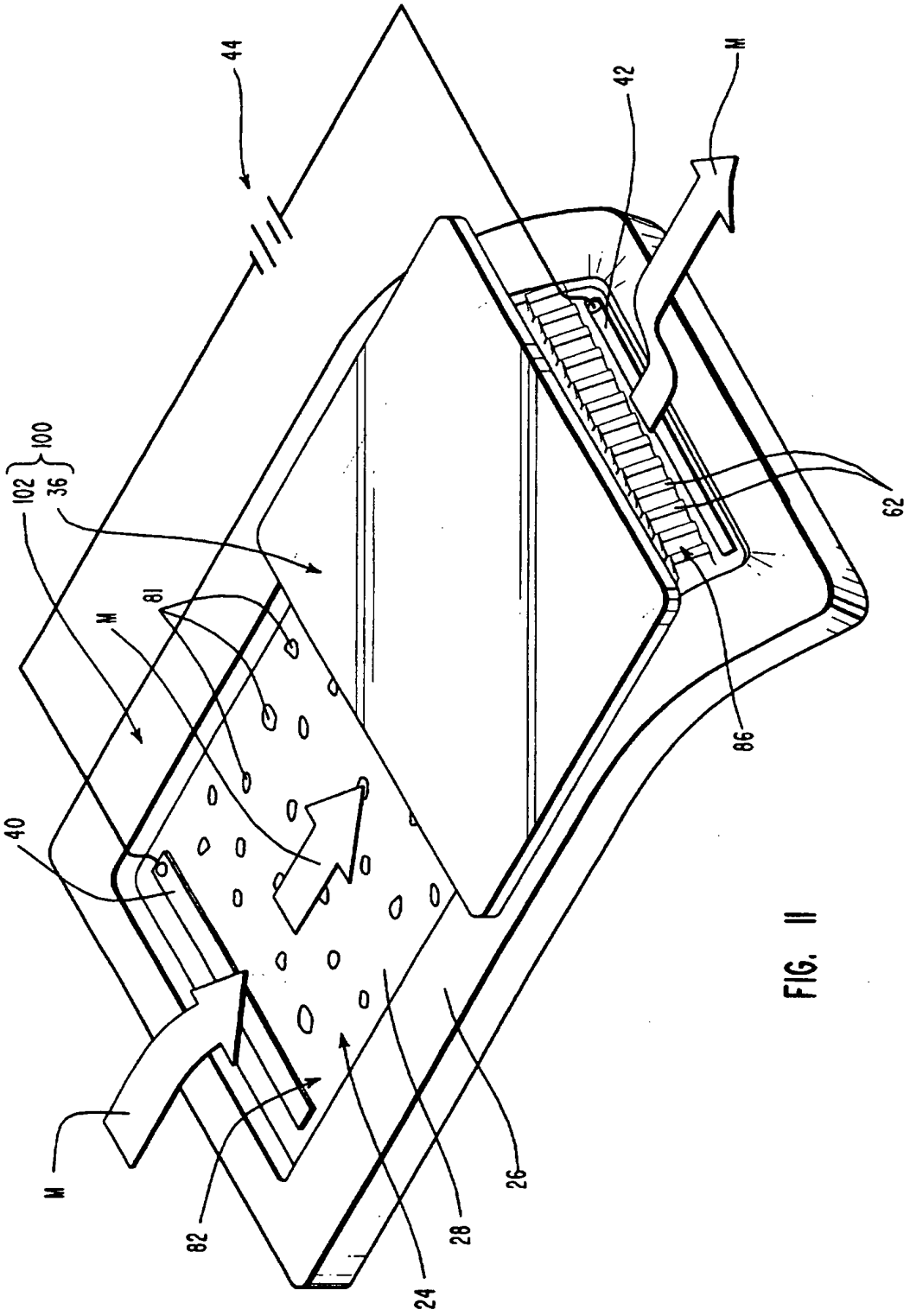

FIG. 11

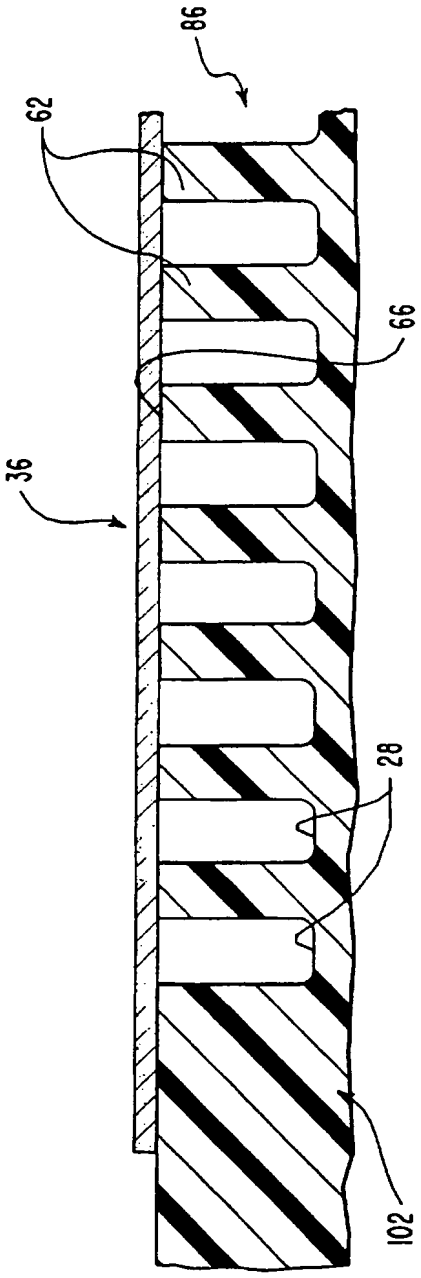

FIG. 12

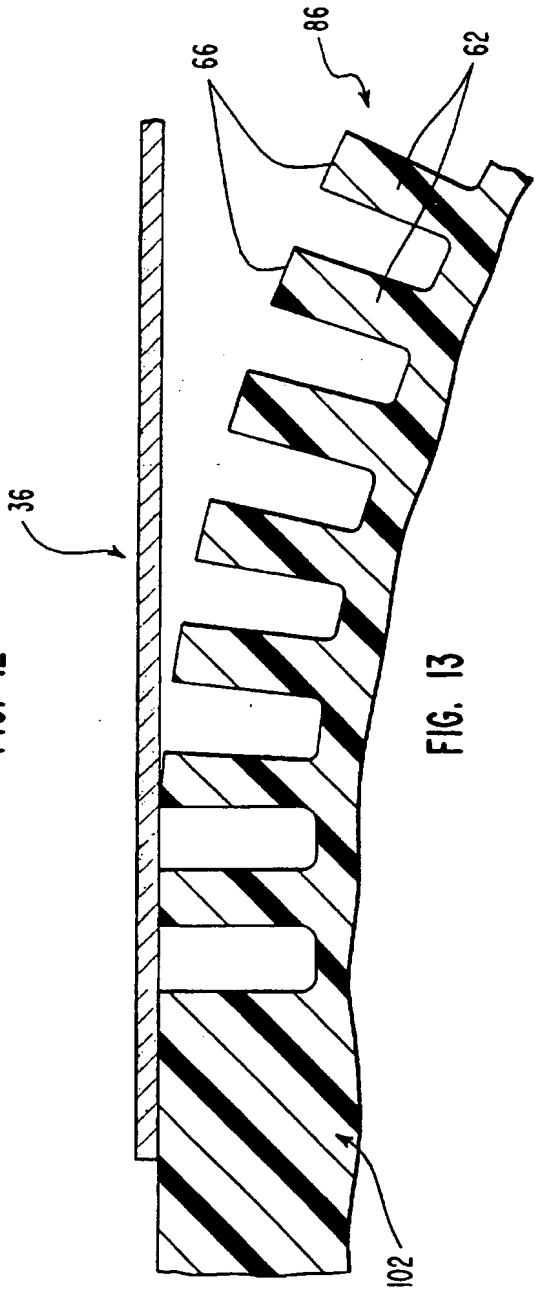

FIG. 13

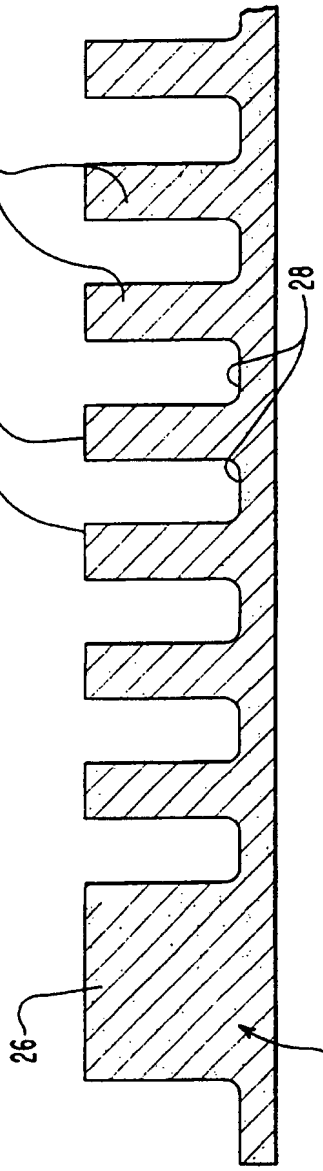

FIG. 14A

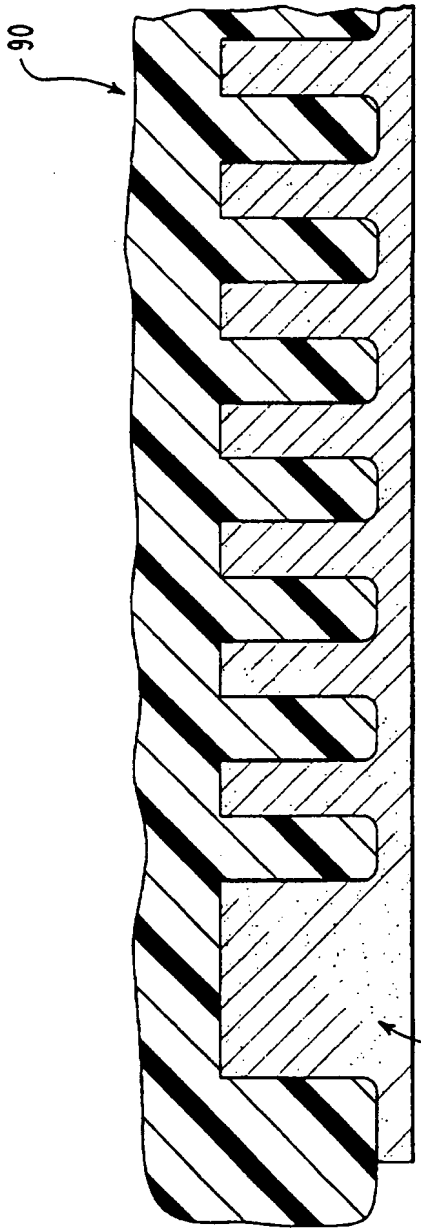

FIG. 14B

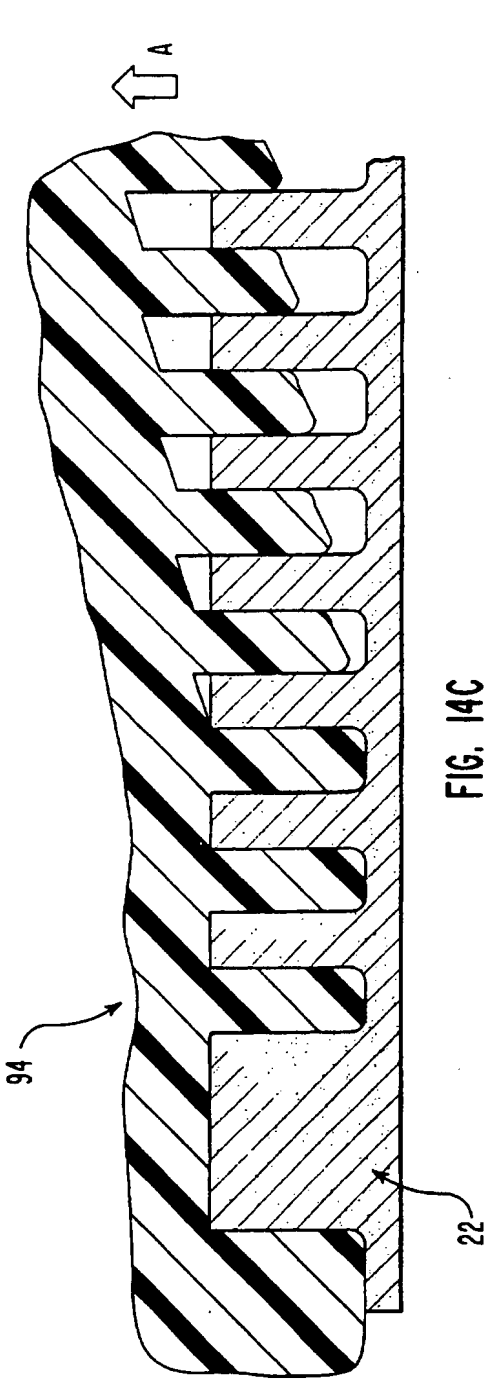

FIG. 14C

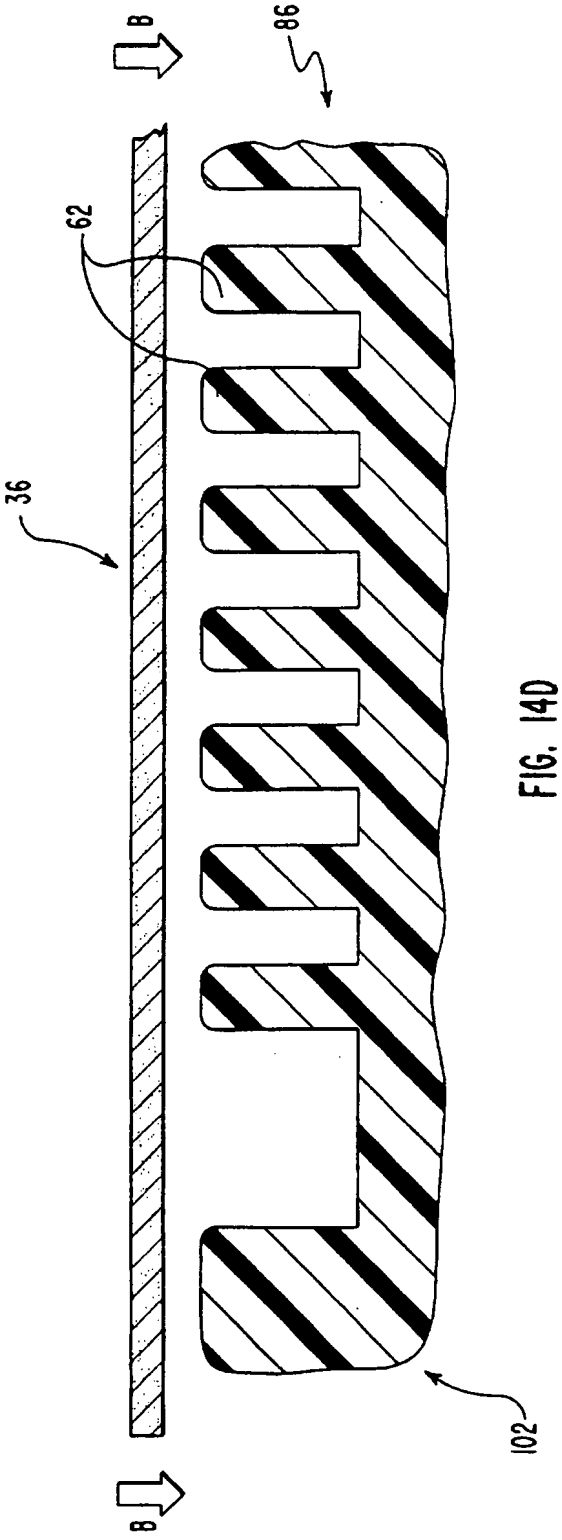

FIG. 14D

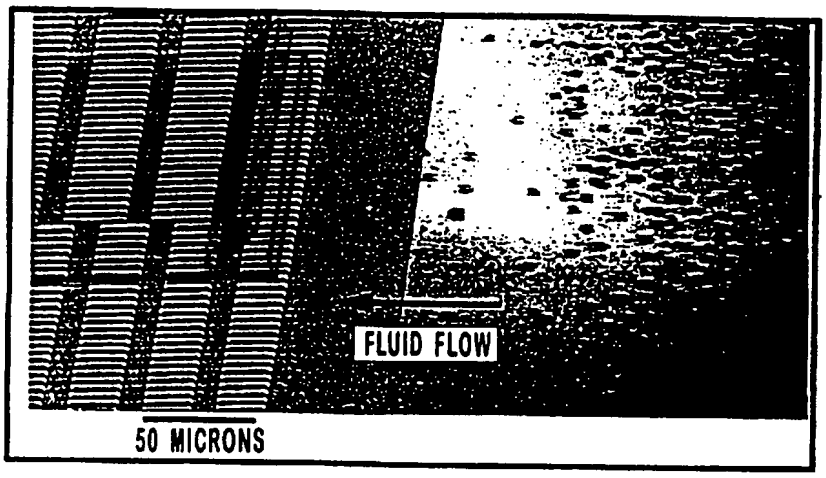

FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15063

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12M 1/00
US CL :435/287.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/283.1, 287.2, 325; 356/344; 422/50, 63, 99; 436/94; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, L	US 5,427,663 A (AUSTIN et al.) 27 June 1995, see entire document.	1-14
A,P	US 5,567,302 A (SONG et al.) 22 October 1996, see entire document.	1
A,P	US 5,549,796 A (CHU et al.) 27 August 1996, see entire document.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 SEPTEMBER 1997	Date of mailing of the international search report 29 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRADLEY L. SISSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15063

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS- File USPAT

Search terms: apparatus, dna, rna, nucleic acid?, polynucleotide?, silicone, dimethylsiloxane, electrophoresis, array